Structural Studies of Iron(III) Complexes of the New Iron-binding Drug, Pyridoxal Isonicotinoyl Hydrazone

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Introduction

Considerable effort is currently devoted to the development of chelating agents that can safely remove toxic accumulations of metal ions from the body [1–3]. The removal of excess body iron in patients with β-thalassemia presents a particular challenge since treatment with an iron-chelating drug must be continued throughout the lifetime of an individual having this genetic disorder [4]. The chronic toxicity, efficacy, convenience of administration and expense of the drug all therefore assume heightened importance [5] and since the only agent presently available, desferrioxamine B, is unsatisfactory in a number of these respects, the search for a more acceptable drug has continued [6, 7]. One of the most promising candidates, identified only recently, is the isonicotinoyl hydrazone of pyridoxal (PIH). This compound removes iron from reticulocytes [8, 9], Chang cells [7] and ferritin [7] *in vitro* and is able to induce substantial iron excretion in rats [10–12]. Although the biological properties of PIH are becoming well documented, little is known of the coordination chemistry underlying interactions between this ligand and iron(III). Since such chemical information will be important in understanding the *in vivo* behavior of this drug, we have undertaken the synthesis and characterization of iron(III) complexes of PIH. This report presents X-ray crystallographic data for two such complexes, one 5- and one 6-coordinate.

Experimental

Red-brown crystals of $[Fe(C_{14}H_{14}N_4O_3)Cl_2]Cl$ (hereafter *A*) which contain the five coordinate complex were obtained directly from methanol solutions with equal molar amounts of $FeCl_3 \cdot 6H_2O$ and PIH present. No base was added to the solution and the mother liquor from which the crystals deposited was highly acidic (pH reading ≅ 0.5). MW = 448.5, space group $P\bar{1}$, $Z = 2$, $a = 12.709(4)$, $b = 8.652(3)$, $c = 8.821(3)$ Å, $\alpha = 105{,}42(2)°$, $\beta = 89.90(2)°$, $\gamma = 107.64(2)°$, V = 888 Å^3. For 2951 reflections with $|F_c|$ or $F_{obs} \geqslant 2\sigma(F)$, R = 0.095, $R_w = 0.056$. Crystals of the six coordinate complex $[Fe(C_{14}H_{14}N_4O_3)Cl_2 \cdot H_2O]Cl \cdot H_2O$ (hereafter *B*) which were also red-brown, were obtained from aqueous solution by dissolving *A* in water and adding NaCl until solid began to form. Here again the mother liquor was highly acidic (pH ≅ 2). MW = 484.5, space group $P2_1/c$, $Z = 4$, $a = 15.358(4)$, $b = 7.304(3)$, $c = 17.442(4)$ Å, $\beta = 101.01(2)°$, $V = 1940$ Å^3. For 1987 reflections with $|F_c|$ or $F_{obs} \geqslant 2\sigma(F)$, R = 0.081, $R_w = 0.058$. Intensity data out to 2θ values of 53° for *A* and 56° for *B* were obtained by $\theta/2\theta$ scans on a FACS-1 Picker automatic diffractometer with Nb-filtered Mo K_α radiation. Full details of the structural analysis will be published elsewhere.

Discussion

In both crystals PIH acts as a neutral, planar, tridentate ligand (Figs. 1 and 2) and the Cl^-: Fe(III) ratio is three. In each case two chloride ions are bonded to the iron (Table I) and the remaining chloride ion is merely a counter-ion, being hydrogen

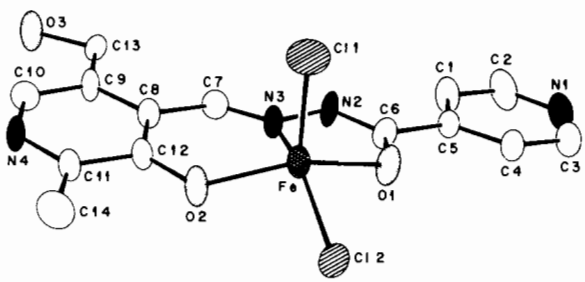

Fig. 1. ORTEP drawing of the $[Fe(PIH)Cl_2]^+$ cation of compound *A*. The chloride counter-iron has been omitted for clarity

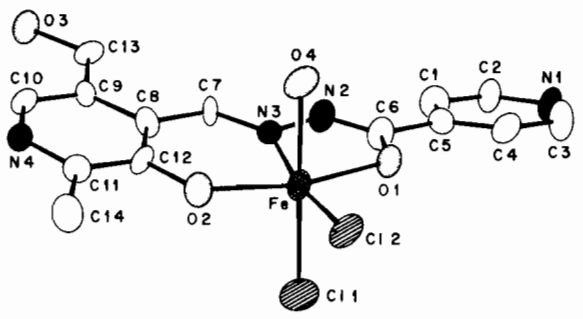

Fig. 2. ORTEP drawing of the $[Fe(PIH)Cl_2H_2O]^+$ cation of compound *B*. The chloride counter-ion and a water molecule, neither of which are coordinated to the Fe(III) atom, have been omitted for clarity.

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TABLE I. Selected Bond Distances, in Å.

	A	*B*
Fe–Cl_1	2.216(3)	2.373(3)
Fe–Cl_2	2.240(3)	2.278(2)
Fe–O_1	1.980(5)	2.018(6)
Fe–O_2	1.918(5)	1.918(6)
Fe–O_4	–	2.151(7)
Fe–N_3	2.137(7)	2.122(6)

bonded to N_1, N_4 and O_3 of three adjacent ions in *A* and to N_1, N_4 and O_4 of adjacent ions in *B*. The coordinated ligand retains the neutrality characteristic of its free form by a transfer of protons from the phenolic oxygen atom (O_2) and the hydrazidic nitrogen atom (N_2) to the pyridine nitrogen atoms N_1 and N_4 [13]. The hydrazidic linkage of the free PIH is neutral and ideally represented as $C_7{=}N_3{-}N_2H{-}C_6{=}O_1$ [14] whereas that of the complexed PIH is, as expected, uninegative and represented as $C_7{=}N_3{-}N_2{=}C_6{-}O_1^-$ [15]. Given that this latter unit is planar and conjugated in both *A* and *B* and in similar complexes [16], it may safely be assumed that folded forms of coordinated PIH are energetically unfavorable and thus unlikely [9]. Given that *A* and *B* are formed from solutions of high acidity, it is clear that O_2 and N_2 are readily deprotonated in the presence of iron (III), as is not unexpected. It seems likely that the tridentate Fe–PIH linkage is independent of pH over a wide range, limited on the low side by reprotonation and on the high side by intrusion of OH^- and eventual formation of hydrous iron(III) oxide.

Although these structures clearly establish the manner in which one PIH binds to a single iron(III) atom, many questions remain as to the precise nature of species formed when it chelates iron *in vivo*. When PIH is administered to rats, the bile flow becomes deep red-brown in color and contains iron in concentrations up to ~80 μg/ml [12]. These observations are consistent with (although not proof of) the formation *in vivo* of an iron–PIH complex. However, the solubility of this complex in bile far exceeds the solubility of any product we have been able to obtain from aqueous solutions containing only PIH and an iron(III) salt at neutral pH. Such solutions invariably give rise to highly insoluble brown precipitates, irrespective of the charging stoichiometry, although soluble complexes can be obtained with the phosphorylated analog, pyridoxal-5-phosphate isonicotinoyl hydrazone. We therefore suggest that iron excreted in response to PIH administration may not necessarily take the form of a simple 2:1 PIH:Fe complex, as has been assumed [9, 11]. Instead, such excretion may involve complexes analogous to *A* and *B* in which the remaining coordination sites of an Fe–PIH moiety are occupied by endogenous molecules (polypeptides, bile acids [17], sugars, *etc.*) and/or complexes in which the ligand has been phosphorylated *in vivo*.

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